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# Dynamics of spread of intestinal colonization with extended-spectrum beta-lactamases in *E.coli*: a mathematical model

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# 1 Introduction

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to 3rd generation cephalosporins. The prevalence of ESBL producing Enterobacteriaceae has increased drastically since it was first discovered in 1983 in Germany. The increasing prevalence of ESBL is of major concern as it is associated with failure of treatment, prolonged hospitalization and increased costs (Helfand and Bonomo, 2006; Rodriguez-Bano et al., 2006; Collignon and Aarestrup, 2007). In addition, ESBL resistant bacteria often carry co-resistance to other antibiotics, further complicating the treatment of infections (Rodriguez-Bano et al., 2006). In other areas of antibiotic resistance, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Bootsma et al., 2006) mathematical models have shown to be a strong tool for interpreting the resistance dynamics and investigating possible interventions. In this report we will attempt to develop such a model for ESBL.

The dynamics of ESBL are very complex and differs from MRSA dynamics by the acquisition routes and type of bacteria carrying the resistance. Different types of ESBL have been identified which can be produced by different bacterial species. Until around 2000 mostly ESBL of type TEM/SHV was found in Europe. In Holland the first ESBL of type CTX-M was detected in 1995 (Hall et al., 2002). Before the introduction of CTX-M the ESBLs reported were predominately stemming from *Klebsiella pneumonia* (Paterson et al., 2003). The CTX-M enzyme is most frequently associated with *Escherichia coli*, which has caused a switch from *K.pneumoniae* to *E.coli* as the most predominant species among ESBL producers (Markovska et al., 2008). Moreover, ESBL *K. pneumonia* is mainly nosocomial acquired, whereas ESBL-producing *E.coli* is also found in strictly community acquired infections (Cantón et al., 2008; Rodriguez-Bano et al., 2006).

In this study we suggest a new mathematical model to describe the ESBL dynamics. The main objective of the study is to investigate the plausibility of different transmission routes by comparing a mathematical model for the spread of ESBL with data for ESBL prevalence. For instance, the increase in CTX-M ESBL prevalence may be due to horizontal transfer of CTX-M between species (Markovska et al., 2008; Cantón et al., 2008). Other authors have shown the importance of travellers returning from holiday with an ESBL bacteria colonization (Laupland et al., 2008; Pitout et al., 2004). The model will therefore include these routes of horizontal transfer and external acquisition of ESBL together with cross-transfer and mutation. These pathways will be discussed in details in the next section. It has been argued that the use of 3rd and 4th generation cephalosporins in animals has an influence on the increase of ESBL prevalence in humans, as the drug resistance may spread via food or other sources like the ground water (Collignon and Aarestrup, 2007). However, this effect is not included as it would result in an overparametrization of the model due to lack of data.

# 2 Model

Our model describes the spread of intestinal colonization with ESBL. Intestinal colonization is considered, because colonization usually precedes clinical infections (Harris et al., 2007a). Moreover, most colonized patients do not develop overt infections and, hence, it is believed that colonization is more important for the spread than clinical infections (Harris et al., 2007b). The model considers a hospital and its catchment area and consist of two levels of dynamics: 1) The flow of patient; and 2) The flow of bacteria and resistant genes. Each of the two levels of dynamics is described in the following sections.

## 2.1 Flow of patients

Hospitalized patients are divided into high-risk and low-risk wards for the acquisition of resistance. Hospital-based studies have suggested a number of risk factors for the acquisition of ESBL including intensive care unit (ICU) admission, antibiotic usage, and mechanical devices (Laupland et al., 2008; Cantón et al., 2008). We identify high-risk wards (ICU, Surgery, Hematology, and lung diseases) as wards at the University Medical Center Utrecht (UMCU), the Netherlands with a high level of ESBL colonized patients in 2008. This classification of high-risk wards is in agrement with previous studies (Coque et al., 2002).
We hypothesize that frequent readmitted patients have a role in maintaining a high ESBL prevalence in the hospital. Similar to Cooper et al. (2004) we therefore allow the probability per unit time to be readmitted to decrease with the time since the most recent hospital discharge. This is modelled by letting discharged patients move first to core groups and from here on into a catchment population with a lower hospitalization rate. Hence, patients discharged from the low- and high-risk wards are separated in two different compartments (core-groups). This flow of patients can be seen in Figure 2.1. Additionally, persons can be removed from the community, either because they die or because they move to another municipality. This is implemented in the model by a constant removal rate from the catchment population and the core groups. As soon as a person is removed, it will be replaced by a new person in the catchment population, which is not colonized with resistant bacteria.

## 2.2 Flow of bacteria

Election of colonization states for the model and the routes of transfer between the states is a fine balance between keeping the model simple and including all important states and routes. The extent of the model is further limited by available information about prevalence and rates. ESBL strains are most often found in *E.coli* (EC) and other Enterobacteriaceae (EB) such as *K. Pneumo-*

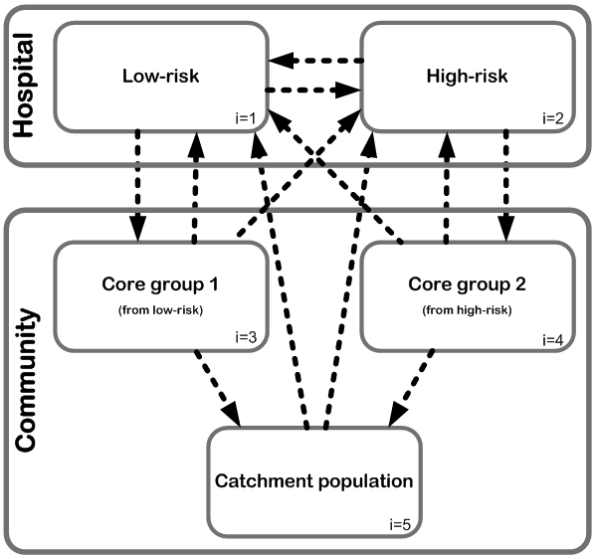
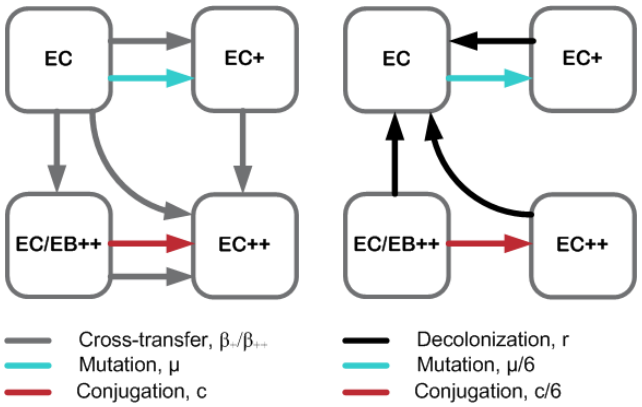



**Figure 2.1:** A sketch of the patient and people flow in the model. The indexes $i$ will be used to refer to the compartments.

*nia* (Romero et al., 2007; Caccamo et al., 2006). For this model we therefore consider EC and another EB type with special focus on the EC population. EC can be ESBL positive of type TEM/SHV (+) or CTX-M (++), which are the dominant types of ESBL. EB is included to be able to incorporate conjugation between species as one of the transfer mechanisms, and therefore the inclusion of EB++ is very interesting for the model. Assuming each individual carries EC, we distinguish four intestinal colonization states, EC, EC+, EC++ and EC/EB++. The population in each hospital ward and community compartment is divided into these four states (Figure 2.2(a) and 2.2(b)). The TEM/SHV phenotype can be obtained by cross-transmission, and mutation whereas the CTX-M phenotype can be obtained by cross-transmission, conjugation and externally from travellers. The model is constructed on the level of human individuals, and the number of bacteria in each individual is not modelled. Whenever a person acquires resistance, the bacteria is assumed to be present in sufficient numbers for the individual to be able to transfer the resistance. Cross-transmission is dependent on the amount of people in the ward with the specific bacteria species and resistance type. Cross-transmission is therefore modelled with the mass action expression $\beta C/N$, where $\beta$ is a constant which is specific for a given bacteria species and resistant type, $C$ is the amount of colonized people with the given bacteria species in the ward of interest and N is the total size of the ward. When conjugation happen from a EB++ patient it is no longer registered in the model to carry EB++. To avoid the need of an extra compartment the cross-transfer of EB++ is based on the amount of people colonized with EB++ as well as EC++. Acquisi-




(a) Hospital (b) Community

**Figure 2.2:** Model describing the transfer of ESBL in *E. coli* (EC) and other Enterobacteriaceae (EB). The model includes the dynamics of the transfer of EC of type TEM/SHV (EC+), EC of type CTX-M (EC++), and EB of type CTX-M (EB++) in the hospital and community. In the hospital the rate of cross-transfer, mutation and conjugation is taken to be three times higher in the high-risk wards, than in the low-risk wards.

tion of ESBL by mutation is independent of the colonization status of other people in the ward. It occurs with a constant rate, $\mu$. Conjugation can occur with a constant rate $c$, when a patient is colonized with EB++. The rates $\beta$, $\mu$ and $c$ at which acquisition happens are assumed to be 3 times lower in the low-risk wards as compared to the high-risk wards. Cross-transmission is disregarded in the community, whereas mutation and conjugation occur with half the rate in the community as compared to the low-risk ward. After the year 2000 there is an extra inflow of EC++ and EB++ to the catchment population from travellers carrying the strains home from holiday. This inflow is assumed to occur to the core group 2 for EC++ and EB++ and to the catchment population for EC++ (Pitout et al., 2004). In the community colonization is lost with the rate $r$ (recovery rate). The decolonization rate is the same for all community groups. Whenever possible the model parameters are found in the literature and the remaining parameters will be estimated. A description of the parameter estimation is given in Section 3.2.
The model is simulated as a discrete stochastic model in `R` (R Development Core Team, 2009) using the fixed-increment time advance method. For each day the following steps are carried out

- transfer between bacteria colonization states within each hospital ward or community group.
- movement of people within the hospital and community as well as hospital admittance and discharge.

- inflow of resistant strains from travellers.

The two first steps are computed by sampling from a Multinomial distribution, as more events can happen to one population during one time interval. The last step is computed by sampling from a Poisson distribution.

# 3 Parameter estimation

## 3.1 Patient flow

Data from the UMCU from 2005 to 2008 with time of admission, discharge and movement within the hospital, including ward specification, is used to estimate the parameters for the patient flow.

### Survival analysis

Part of the data for the time between two hospital admissions is censored, and survival analysis is therefore used to calculate the time to readmission. In this context, censored data means that only the time between discharge and the end time of the available data set is know. Thus, only a minimum time between readmissions is known, but there is no observed readmission. For other patients two subsequent admissions are registered in the data set. Thus, for these patients the actual readmission time is known. These readmission times are also called un-censored.
People discharged from the hospital can be readmitted from either the core-group or catchment population as sketched in Figure 3.1. The survival func-

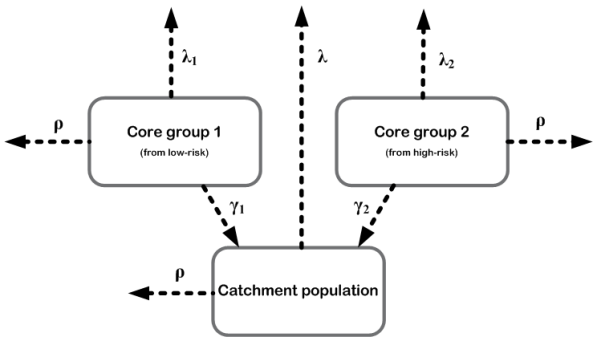



**Figure 3.1:** A sketch of the simplified patient flow from with the survival function for readmission time (Equation (3.4)) is calculated. $\lambda_1$, $\lambda_2$ and $\lambda$ are readmission rates and $\gamma_1$ and $\gamma_2$ are the rates by which patients are moved to the catchment population. The removal rate, $\rho$, is fixed to one over the mean time of stay in the same municipality, which in the Netherlands is 21.6 years.

tion $S_i(t)$, for readmittance after discharge from the hospital compartment $i$ is calculated as

$$S_i(t) = P_i(T > t) = P_i(T = \infty) + P_i(\infty > T > t) \tag{3.1}$$

where the first term corresponds to patients who are never readmitted, i.e., removal from the extramural population, and the second term to a readmission

at least a time $t$ after the previous admission.

$$P_i(T=\infty) = \int_0^\infty \rho \exp(-(\rho+\lambda_i+\gamma_i)\tau)d\tau + \int_0^\infty \gamma_i \exp(-(\rho+\lambda_i+\gamma_i)\tau)\rho\exp(-(\rho+\lambda)\tau)d\tau \quad (3.2)$$

and

$$P_i(T>\infty>t) = \int_t^\infty \lambda_i \exp(-(\rho+\lambda_i+\gamma_i)\tau)d\tau + \int_t^\infty \left(\int_0^\tau \gamma_i \exp(-(\lambda_i+\gamma_i+\rho)\tau')\lambda\exp(-(\lambda+\rho)(\tau-\tau'))d\tau'\right)d\tau \quad (3.3)$$

By solving the integrals the survival function is found to be

$$S_i(t) = \frac{\rho}{\rho+\lambda_i+\gamma_i} + \frac{\rho\gamma_i}{(\rho+\lambda)(\rho+\lambda_i+\gamma_i)} + \frac{\lambda_i}{\rho+\lambda_i+\gamma_i}\exp(-(\rho+\lambda_i+\gamma_i)t) - \frac{\lambda\gamma_i}{(\lambda_i+\gamma_i-\lambda)}\frac{1}{(\rho+\lambda_i+\gamma_i)}\exp(-(\rho+\lambda_i+\gamma_i)t) + \frac{\lambda\gamma_i}{(\lambda_i+\gamma_i-\lambda)}\frac{1}{\lambda+\rho}\exp(-(\lambda+\rho)t)\ , \quad (3.4)$$

where the readmission rate $\lambda_i$ and the rate of movement to the catchment population $\gamma_i$ are different for patients discharged from the low-risk (i=1) and high-risk (i=2) wards. The readmission rate from the catchment population, $\lambda$ is the same, independently of which ward a patient was discharged from at last hospitalization. The removal rate, $\rho$, is assumed to be the same in the whole community and is kept fixed in the estimation. A person is removed from the catchment population or core groups after a mean of 21.6 year, which corresponds to the mean time that persons stay in the same municipality in the Netherlands. This is considered to be a good estimate of the time period in which people might be readmitted to the same hospital. The other parameters of the survival function are determined by Maximum Likelihood estimation, where the log-likelihood function is

$$\log(L) = \sum_{\text{i} \in \text{ uncensored data}} \log\left(\frac{f_i(t_i)}{S_i(t_i)}\right) + \sum_{\text{i} \in \text{ all data}} \log(S_i(t_i))\ , \quad (3.5)$$

and $f(t) = -dS(t)/dt$. The likelihood function is optimized in `R` using the `optim` function (R Development Core Team, 2009).

## Results

The suggested survival function gives a good fit to the data for readmission as seen in Figure 3.2. It should be noted that the survival function does

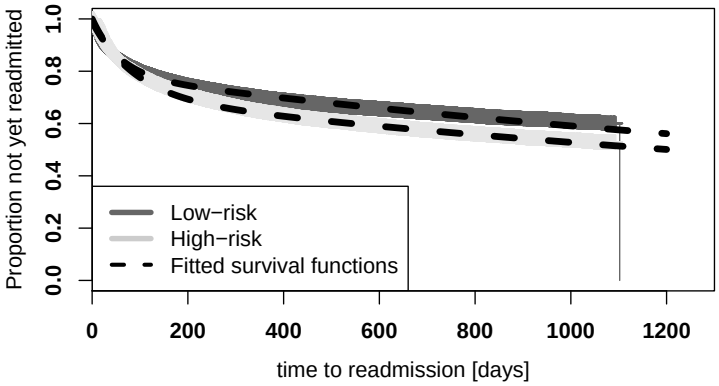



**Figure 3.2:** Fit of the survival function for people discharged from high-risk and low-risk wards at University Medical Centre Utrecht. The data used is from 2005 to 2008. High-risk wards are ICU, Surgery, Hematology and lung diseases, which are identified as those with a high risk of colonization with ESBL carrying bacteria.

not describe *to* which ward the patients are readmitted. It only states what the readmission time is when discharged *from* a specific ward. Based on the UMCU patient data we find the percentage of patients discharged from the low- or high-risk wards, which are readmitted to either of these wards, and from here the readmission rates of the core-groups are calculated.
The readmission rate from the catchment population to each hospital ward and the size of the catchment population are estimated, such that the size of the hospital wards are in agrement with the UMCU data. From the UMCU data the length of stay in each ward, the transfer between wards, and the fraction of patients which dies at the hospital are also deduced. As soon as a person is removed from the population a new person, colonized with non ESBL *E.coli*, is assumed to appear in the catchment population. All parameters for the patient flow are given in Table 3.1 and Table 3.2.

## 3.2 Bacteria flow

The duration of colonization after discharge from the hospital have recently been examined in a study by Apisarnthanarak et al. (2008). They found a median duration of outpatient colonization with ESBL of 98 days, i.e. a mean duration of 141 days. This length of colonization will be used for all ESBL producing bacteria in the model. The inflow of resistance from travellers is initiated in year 2000. The inflow of EC++ and EB++ to core group 2 is fixed to 0.35 per year, and the inflow of EC++ to the catchment population is fixed

**Table 3.1:** Parameters for patient flow calculated from UMCU data with a 95% confidence interval (CI.

| Parameter | Value (95% CI) |
|---|---|
| Mean length of stay: | |
| Low-risk | 5.32 (5.27 - 5.36) days |
| High-risk | 6.68 (6.61 - 6.76) days |
| Mean time to admission: | |
| From Core-group 1 | 247.57 (239.53 - 255.62) days |
| From Core-group 2 | 293.71 (282.29 - 305.23) days |
| From Catchment population | 8.97 (8.63 - 9.30) years |
| Mean time before moving to catchment group: | |
| Core-group 1 to catchment: | 73.88 (70.56 - 77.20) days |
| Core-group 2 to catchment: | 130.55 (122.94 - 138.16) days |

**Table 3.2:** The table shows data values for the percentage of patients that moved within the UMCU, were discharged, or died at the UMCU between 2005 and 2008. The table also contain the percentage of people admitted to each hospital ward from the community.

| To: / From: | Low-risk | High-risk | Discharged | Dead |
|---|---|---|---|---|
| Low-risk | - | 13% | 86% | 1% |
| High-risk | 24% | - | 73% | 3% |
| Core-group 1 | 87% | 13% | | |
| Core-group 2 | 23% | 77% | | |
| Catchment population | 72% | 28% | | |

to 5 per year (Pitout et al., 2004). The unknown parameters for the flow of bacteria are determined in three steps. First in Section 3.2 three values of the cross-transfer rate of EC+ are determined such that the basic reproduction number equals 0.50, 0.75 or 1.00. Secondly for each of the estimations of the cross-transfer rate, the mutation rate for EC+ is estimated based on the prevalence of ESBL in The Netherlands before the large increase in ESBL caused by CTX-M. The prevalence of EC+ is assumed to have reached an equilibrium level before the introduction of CTX-M. The used prevalence data is from the study of Stobberingh et al. (1999) which found that the ESBL prevalence at Dutch hospitals in 1997 was 0.35% for EC+. Finally, the parameters for the transfer of EC++ are estimated using nosocomial ESBL prevalence data deduced from the Dutch EARSS data (2000-2008). The EARSS prevalence is based on aggregated data for all of the Netherlands. The parameter estimation of the model can therefore be based on the mean of several simulations. Thus, estimation based on the ESBL prevalence is made by minimizing the least squares (LS) value of the difference between the simulated mean yearly

ESBL prevalence based on up to 50 simulations and the data. Due to the stochasticity of the model the LS value will be noise even when taking the mean over several simulations. Therefore the LS estimation will be carried out using a simultaneous perturbation stochastic approximation (SPSA) algorithm explained in Section 3.2.

### Reproduction Number

The basic reproduction number, $R_0$, is the mean number of secondary colonizations that one colonized individual will cause before it get decolonized. This number is often used in epidemiology, as it can help determine whether an infection (or in this case colonization) will spread in a population. If $R_0 < 1$ the infection will die out, and if $R_0 > 1$ the infection can spread in a population. Alternatively the single readmission number, $R_A$, can be calculated, which is the mean number of secondary colonizations that one colonized individual will cause during one hospital admissions. We calculate $R_0$ and $R_A$ for a simplified situation where a person can be either susceptible (i.e. in the category EC) or colonized with EC+. In this way the initial colonization of EC+ bacteria can be studied. We only calculate the reproduction number for cross-transmission, hence the only way of acquiring EC+ is by cross transfer in the hospital with rate $\beta$ in the high-risk wards and $\beta/3$ in the low-risk wards. The colonization can be lost in the community with the rate $r$.
$R_0$ and $R_A$ are found as the largest eigenvalue for the next-generation matrix $\boldsymbol{K}^0$ and $\boldsymbol{K}^A$, respectively (Diekmann and Heesterbeek, 2000). Each element of $\boldsymbol{K}$, $k_{ij}$ is the expected number of new colonized people in compartment $i$ caused by one person colonized in compartment $j$ either during one hospitalization ($R_A$) or during the whole duration of the colonization ($R_0$). The expected number of new colonized people in compartment $i$ is given by the transfer rate $\beta$ multiplied with the time, $u$, spend in this compartment before discharge from the hospital or loss of colonization. The movement between compartments can be considered as a Markov jump process containing each of the transient states and a compartment representing the absorbing state, i.e. discharge of a patient or loss of colonization. The process has an intensity matrix of the form

$$\Lambda = \begin{bmatrix} \boldsymbol{T} & \boldsymbol{t} \\ \boldsymbol{0} & 0 \end{bmatrix} \tag{3.6}$$

where $\boldsymbol{T}$ describes the rate of movement between the transient states and $\boldsymbol{t}$ contains the rates by which exit to the absorbing state takes place. The probability of going from state $i$ to $j$ is written as $p_{ij}$, for example the probability of going from core group 1 to the low-risk ward is

$$p_{31} = \lambda_{31}/(r + \gamma_1 + \rho + \lambda_{31}) \,, \tag{3.7}$$

where $\lambda_{31}$ is the readmission rate from core-group 1 to the low-risk ward, $r$ is the decolonization rate, $\gamma_1$ is the rate of transfer from core group 1 to the catchment population, and $\rho$ is the removal rate of persons from the population. If we apply this notation to the model for the patient flow shown in Figure 2.1, we can write down the complete $\boldsymbol{T}$ matrix for the case where discharges from the hospital are considered as the absorption state

$$\boldsymbol{T}^A = \begin{bmatrix} -1/T_1 & p_{12}/T_1 & p_{13}/T_1 \\ p_{21}/T_2 & -1/T_2 & p_{23}/T_2 \\ p_{31}/T_3 & p_{32}/T_3 & -1/T_3 \end{bmatrix} \tag{3.8}$$

or when loss of colonization is considered as the absorption state

$$\boldsymbol{T}^0 = \begin{bmatrix} -1/T_1 & p_{12}/T_1 & p_{13}/T_1 & p_{14}/T_1 & 0 & 0 \\ p_{21}/T_2 & -1/T_2 & p_{23}/T_2 & 0 & p_{25}/T_2 & 0 \\ p_{31}/T_3 & p_{32}/T_3 & -1/T_3 & p_{34}/T_3 & 0 & 0 \\ p_{41}/T_4 & 0 & p_{43}/T_4 & -1/T_4 & 0 & p_{46}/T_4 \\ 0 & p_{52}/T_5 & 0 & 0 & -1/T_5 & p_{56}/T_5 \\ p_{61}/T_6 & p_{62}/T_6 & p_{63}/T_6 & 0 & 0 & -1/T_6 \end{bmatrix} \tag{3.9}$$

In both cases $1/T_i$ is the mean length of stay in the given compartment $i$, as shown in Figure 2.1. The time until absorption $\tau$ is said to have a phase type distribution $PH(\pi, \boldsymbol{T})$, where $\pi$ is the initial distribution. It can be shown that if $\boldsymbol{U} = (-\boldsymbol{T})^{-1}$, then each element $u_{ij}$ of the matrix $\boldsymbol{U}$ is the expected time spent in state $j$ given initiation in state $i$ prior to absorption. In this way each element in the next generation matrix $\boldsymbol{K}$ can be found as

$$\boldsymbol{K} = (-\boldsymbol{T})^{-1}_{ij}\beta_j = u_{ij}\beta_j \ , \tag{3.10}$$

where $\beta_4 = \beta_5 = \beta_6 = 0$, as there is no cross-transfer in the community. $R_0$ and $R_A$ can then be found as the largest eigenvalue of $\boldsymbol{K}^0$ and $\boldsymbol{K}^A$, respectively

### Stochastic approximation

The bacteria transfer parameters will be determined by LS estimation. Due to the stochasticity of the model the object function will be noisy even when taking the mean over several simulations. We therefore use a stochastic approximation method to find the minimum object function. The computation of each object function is very time consuming as the model has to be simulated repeatedly for several years to obtain a mean value for the prevalence which can be compared with the observations. Therefore it is desirable to keep the number of evaluations of the object function low. When more than one parameter has to be estimated simultaneously as is the case for the transfer of EC++, the Simultaneous Perturbation Stochastic Approximation (SPSA) method can be used. The SPSA method uses only two measurements of the object function to approximate the gradient, whereas the Finite Difference

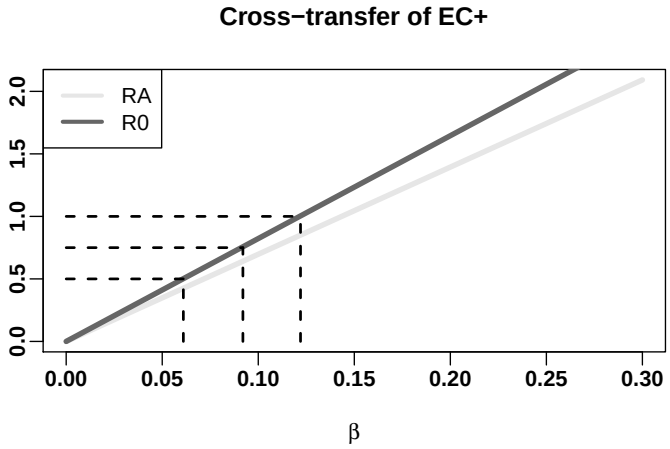
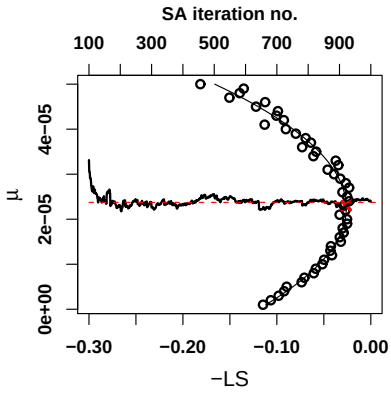



**Figure 3.3:** $R_0$ and $R_A$ for different values of the cross-transfer rate in the high-risk wards. $R_0$ equals 1.00, 0.75 and 0.50 for $\beta_+$ values of 0.122, 0.092, and 0.061, respectively. The decolonization rate is fixed to $1/141$ days$^{-1}$.

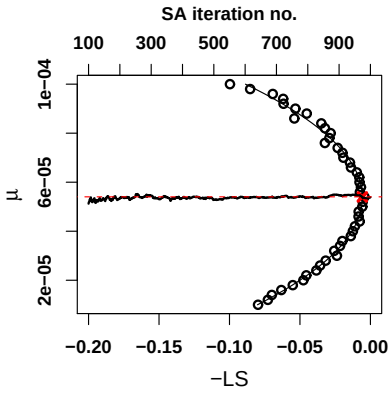
Stochastic Approximation (FDSA) method uses two measurements per parameter. When only one parameter needs to be estimated the SPSA method simply reduces to the FDSA method. For both methods the aim is to find the set of parameters, for which the gradient of the object function equals zero. A good description of the SPSA algorithm and its implementation can be found in Spall (1998).

## Results

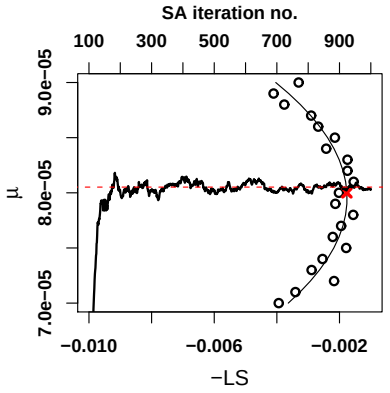
The basic reproduction number, $R_0$ and the single admission reproduction number, $R_A$ are computed in `R` (R Development Core Team, 2009) for difference values of the cross-transmission rate, $\beta$. The result is plotted in Figure 3.3. The cross-transmission rate in the high-risk wards is found to be 0.122, 0.092, and 0.061 in order to give a basic reproduction number of 1.00, 0.75, and 0.50, respectively. The unit for the rates is day$^{-1}$. For the three estimates of the cross-transmission rate, the mutation rate shown in Figure 3.4 is found by stochastic approximation and evaluation of the least squares value. The mutation rates are found to be $2.3 \cdot 10^{-5}, 5.4 \cdot 10^{-5}$, and $8 \cdot 10^{-5}$ for corresponding $\beta$ values of 0.122, 0.092, and 0.061, respectively.
The cross-transfer rate for EC++ and EB++, and the conjugation rate is found by evaluation the LS value for different values of the cross-transfer rate, $\beta_{++}$, and conjugation rate, $c$, as seen in Figure 3.5. The intension was to use SPSA to estimate the parameters, but it has not been possible to find a proper implementation of the coefficients for the SPSA method, and it was therefore




(a) $\beta_+ = 0.122$




(b) $\beta_+ = 0.092$




(c) $\beta_+ = 0.061$

**Figure 3.4:** Estimate of the mutation rate, $\mu$ using a stochastic approximation method to find the parameter region giving the lowest least squares (solid black line). Subsequently, the mean of 50 evaluation of the LS for different rates in this region is computed (open circles), and the minimum is found by fitting a second order polynomial (solid grey line) to these values and finding the minimum. The mutation rate is found to be $2.3 \cdot 10^{-5}, 5.4 \cdot 10^{-5}$, and $8 \cdot 10^{-5}$ (dotted horizontal line) for corresponding $\beta_+$ values of 0.122, 0.092, and 0.061, respectively.

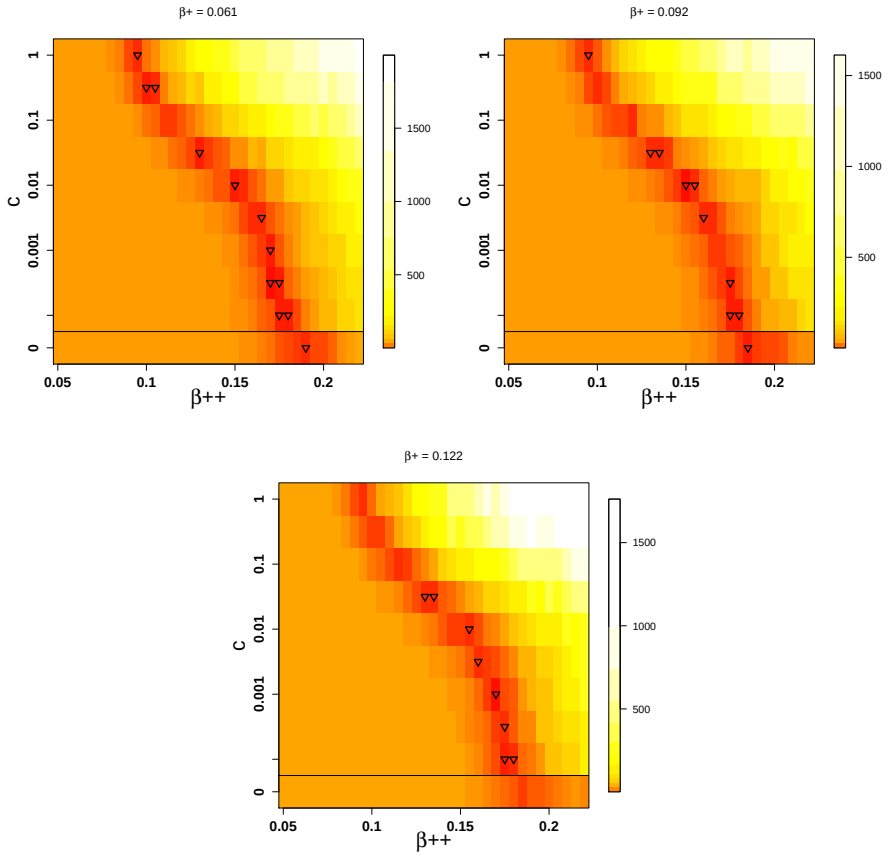



**Figure 3.5:** Least square values (colors) for different combination of the cross transfer rate, $\beta_{++}$, of EC++ and EB++, and the conjugation rate, $c$. Each estimated LS value is the mean of 10 repetitions for which the prevalence is calculated as the mean of 50 simulations of the model. The value of $c$ below the line is 0, whereas it about the line are from $10^{-4}$ to 1. The triangles marks the least square values below 3.

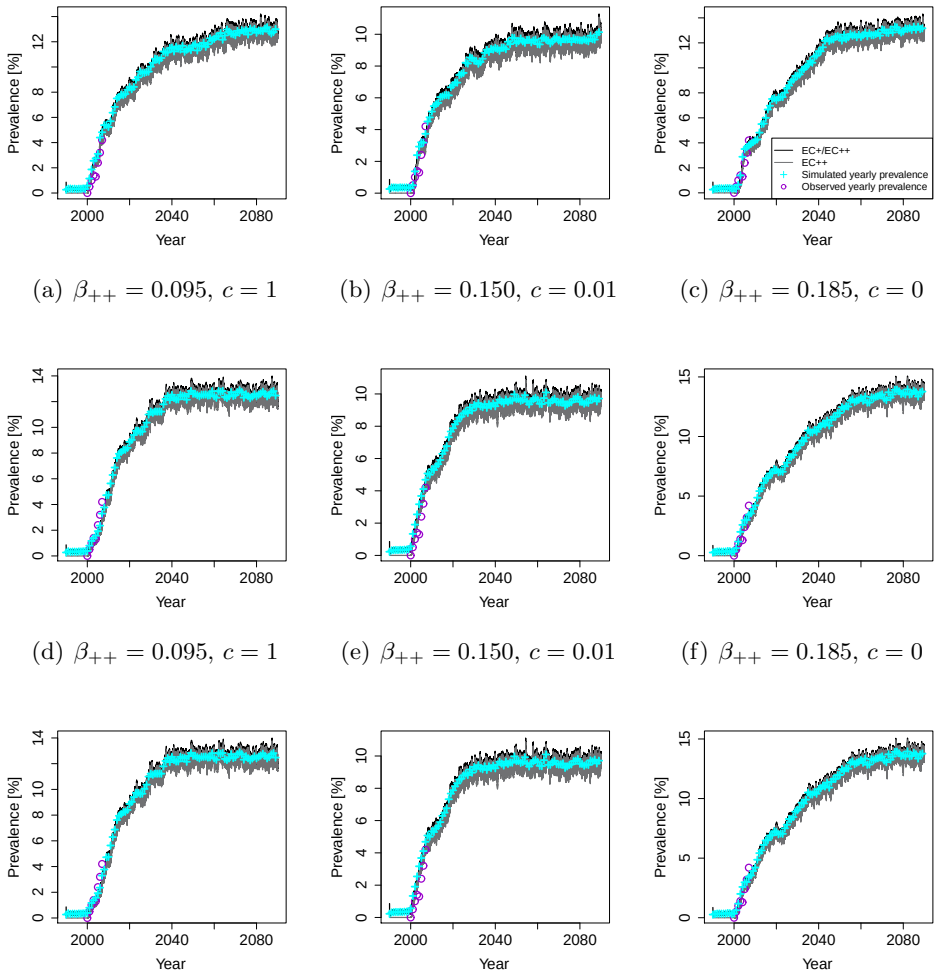
chosen to compute the LS value for a span of model parameter values.
It is seen to be difficult to separate the conjugation rate and the cross-transfer rate. This is caused by the low amount of data available for the estimation, and the implementation of the cross-transfer to the EC/EB++ colonization state in the model. According to the least squares estimation, the conjugation rate can take on values from 0 to 1 per day, whereas the cross transfer can take on values between 0.095 and 0.19 per day.

# 4 Investigating the spread of resistance

The change over time in the total EC+ and EC++ prevalence and the EC++ prevalence is plotted in Figure 4.1, for the three estimates of $\beta_+$ and $\mu$, and for each of these three pairs of $\beta_{++}$ and $c$ values: $\beta_{++} = 0.095$ and $c = 1$, $\beta_{++} = 0.150$ and $c = 0.01$, and $\beta_{++} = 0.185$ and $c = 0$. The model predicts that the total point-prevalence will increase to values between 9.6% and 13.7% depending on the parameter values used. The prevalence in each of the hospital wards from 1990 to 2009 can be seen in Figure 4.2 for one combination of model parameters. The equilibrium prevalence for each of these wards are: low-risk ward 5.0%, high-risk ward 18.1%, core group 1 4.1%, core group 2 14.0%, and catchment population 0.5%. The prevalence in the high-risk ward is thus 3.6 times higher than in the low-risk ward, and similarly the prevalence in core group 2 is 3.4 times higher than the prevalence in core group 1. This is caused by the patient flow, as patients discharged from the high-risk ward is most frequently (77%) also readmitted to the high-risk ward. The last subplot in Figure 4.2 show a close-up of the total estimated yearly mean prevalence for EC+ and EC++ in the hospital until year 2010.

With the parameters used for Figure 4.2 the mean number of patients becoming colonized during one day with either EC+ or EC++ in the high-risk ward in the year 2009 has been calculated. The mean number of occupied beds in the high-risk wards is 183. Out of these patients 0.04 and 0.01 patients per day will be colonized with EC+ due to cross-transfer and mutation, respectively; and 1.92 and 0.16 patients will be colonized with EC++ due to cross-transfer and conjugation, respectively.

The relative importance of each of the three transfer mechanisms: cross-transfer, conjugation, and mutation for the high-risk ward in the year 2009 is plotted in Figure 4. The model predicts that most transfers of EC++ and EC+ will happen due to cross-transfer. Furthermore, the model predicts that a minimum of 57% patients will acquire EC++ due to cross-transfer (for $c = 1$) in the high-risk ward. However, it can not be ruled out that cross-transfer is the only transfer mechanism for EC++ in the hospital, i.e. that $c = 0$.




(a) $\beta_{++} = 0.095$, $c = 1$ (b) $\beta_{++} = 0.150$, $c = 0.01$ (c) $\beta_{++} = 0.185$, $c = 0$

(d) $\beta_{++} = 0.095$, $c = 1$ (e) $\beta_{++} = 0.150$, $c = 0.01$ (f) $\beta_{++} = 0.185$, $c = 0$

(g) $\beta_{++} = 0.095$, $c = 1$ (h) $\beta_{++} = 0.150$, $c = 0.01$ (i) $\beta_{++} = 0.185$, $c = 0$

**Figure 4.1:** The mean of 50 simulations of the model for different values of the model parameters. In the top panel $\beta_+ = 0.061$ and $\mu = 8 \cdot 10^{-5}$, in the middle panel $\beta_+ = 0.092$ and $\mu = 5.4 \cdot 10^{-5}$, and in the bottom panel $\beta_+ = 0.122$ and $\mu = 2.3 \cdot 10^{-5}$. A total prevalence of EC and EC++ between 9.6% and 13.7% is predicted to be reached in year 2030 or later.

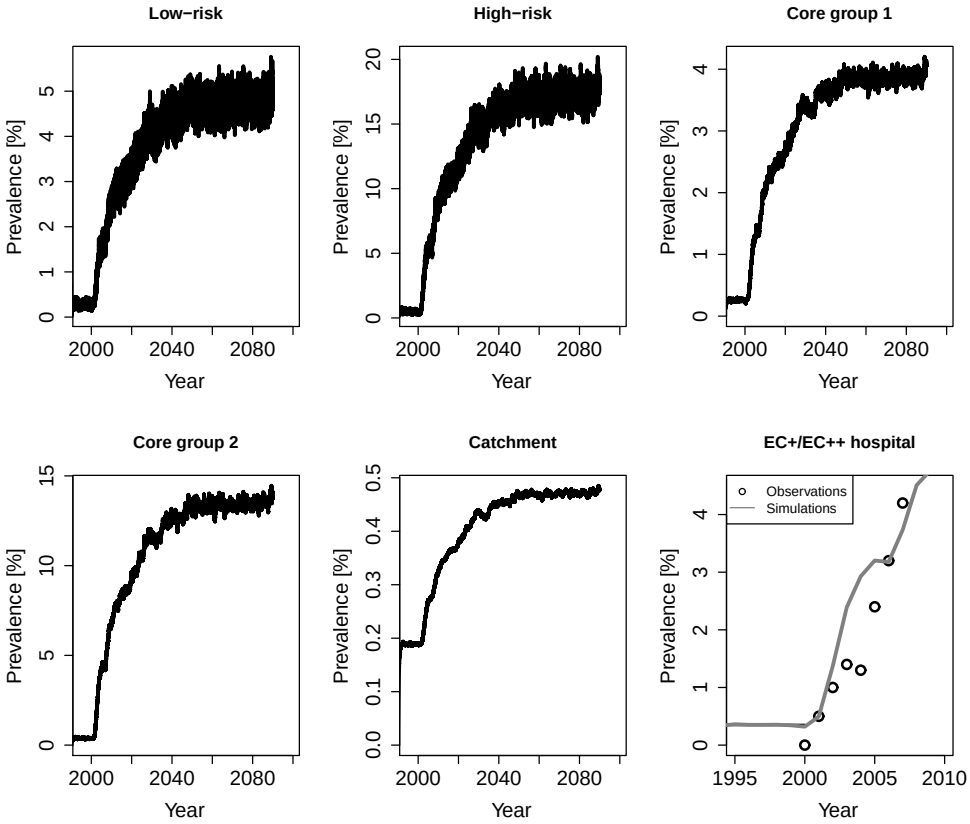



**Figure 4.2:** The total mean prevalence of EC+ and EC++ in each hospital ward and community compartments from 1990 to 2009 for 50 simulations. The parameters used for the simulation was: $\beta_+ = 0.061$, $\mu = 8 \cdot 10^{-5}$, $\beta_{++} = 0.150$, and $c = 0.01$. The plot in the lower right corner show the observed and simulated prevalence EC+ and EC++ prevalence in the hospital.

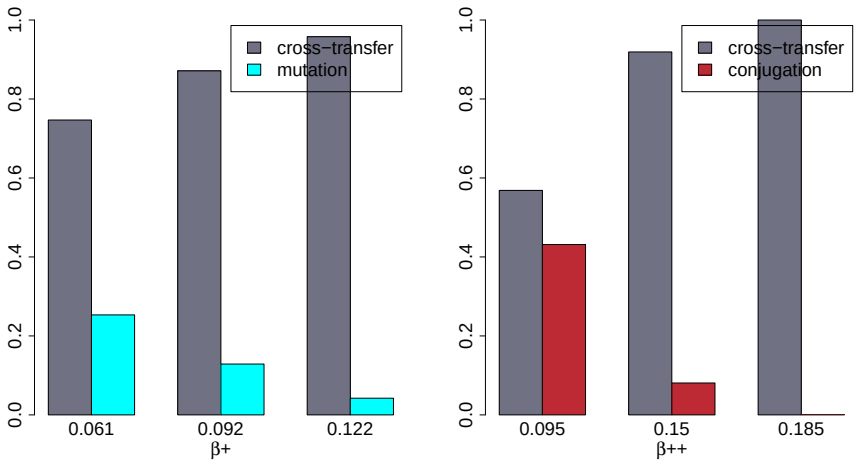



**Figure 4.3:** The relative importance of each of the three transfer mechanisms: cross-transfer, conjugation and mutation, for the transfer of EC+ ($\beta_{++} = 0.150$, $c = 0.01$) and EC++ ($\beta_{+} = 0.061$, $\mu = 8 \cdot 10^{-5}$) as measured in the high-risk ward in year 2009 from 50 simulations of the model.

# 5 Conclusion and outlook

In this study a mathematical model for the spread of ESBL resistant *E.coli* among patients in a hospital and the surrounding catchment population has been introduced and used to described prevalence data from the Netherlands. Several statistical methods have been applied to estimate the model parameters. The patient flow data was studied by survival analysis. This enabled us to get an estimate for the time to readmission when discharged from either the low-risk or high-risk hospital wards. It is hypothesized that readmission plays a role for the spread of resistant bacteria. The high prevalence of EC+ and EC++ colonized patients in the core group with patients discharged from the high-risk ward, indicates that especially patients readmitted to the high-risk wards contribute to the increasing prevalence. It could be interesting in a future simulation study to further investigate the importance of readmission and the effect of different interventions on the prevalence.

There are several theories with regards to the spread of ESBL resistant bacteria, but the actual prevalence data is very sparse. Based on the available data we have developed an adequate model that can explain the increase in prevalence from year 2000. It has not been possible to separate the effect from conjugation and cross-transfer on the ESBL prevalence of type CTX-M, as several combination of the cross-transfer rate and conjugation rate give a good fit to data. However, the model predicts that a minimum of 57% of the acquisition of EC++ colonization is due to cross transfer.

The transfer rates for each hospital and community compartments are all related by a ratio fixed in the model. Due to the high prevalence of resistant bacteria in the high-risk ward, this ward is a central element for estimation of the model parameters. It could therefore be interesting to look at the transfer going on inside the high-risk wards alone. A surveillance study, where the colonization status of all patient in one or two hospital high-risk hospital wards are followed over a couple of month, could be an idea for a better understanding of the transfer mechanisms.

The mean duration of colonization with EC+, EC++ and EB++ after discharge from the hospital has in this study been fixed to 141 days. Whether patients readmitted to the hospital are colonized with resistance bacteria is among other things dependent on the duration of colonization. It would therefore be interesting to investigate the effect of increased or decreased length of colonization by simulation studies. Furthermore the model could be improved, if data from colonization studies of each of the colonization states EC+, EC++ and EB++ were available.

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